



## Age- and sex-dependence of muscle quality: Influence of intramuscular non-contractile tissues

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### ABSTRACT

**Purpose:** Muscle quality is explained by the ratio between muscle size and strength. Conventionally, muscle size is evaluated without considering the composition of contractile and non-contractile tissues in muscle, hence the influence of non-contractile tissues on muscle quality is not fully understood, especially within aging muscle. This study investigated the differences in intramuscular non-contractile tissues between different age and sex groups, and investigated their influence on muscle quality.

**Methods:** Eighty-two older and 64 young females and males participated. Muscle cross-sectional area (quadriceps and hamstrings), separating contractile and non-contractile areas, was calculated from the magnetic resonance image of the right mid-thigh. Maximal voluntary isometric knee extension and flexion torque was measured. Torque/muscle area and torque/contractile area were calculated for each age and sex group.

**Results:** Non-contractile/muscle area was higher in older than in young individuals in both muscle groups ( $p < 0.05$ ), and it was greater in the hamstrings than in the quadriceps. For the hamstrings, torque/muscle area was lower in older than in young individuals in both sexes ( $p < 0.05$ ). However, torque/contractile area did not show the differences between age groups, only between sexes (males > females) ( $p < 0.05$ ).

**Conclusions:** The results indicate that 1) the presence of non-contractile tissues varies by age and muscle groups, 2) the extensive presence of non-contractile tissues can contribute to the underestimation of its muscle quality, and 3) the sex differences in muscle quality are influenced by factors other than muscle composition.

### 1. Introduction

Skeletal muscle size is the main determinant of a muscle's capacity to exert force (i.e., muscle strength). The quadriceps and hamstrings muscles are large muscle groups in the human body and play a crucial role in daily movement and stable posture. Although aging leads to the loss of both thigh muscle size and strength (i.e., sarcopenia), the decline in muscle strength precedes the loss of muscle size. Thus, the ratio between muscle size and strength (e.g., muscle torque per unit muscle area) is widely utilized to indirectly assess muscle quality (Akima et al.,

2001; Francis et al., 2017a; Kent-Braun and Ng, 1999; Metter et al., 1999; Miller et al., 2021; Morse et al., 2004). The decline in muscle quality is often explained by an impairment in neural control (Akima et al., 2001; Morse et al., 2004; Yue et al., 1999). However, the findings on the age-related change in muscle quality is inconsistent, and some studies suggest that other factors also contribute to the decline in muscle quality seen in older people (Akima et al., 2001; Kasai et al., 2015; Metter et al., 1999).

Skeletal muscle is composed not only of muscle fibers (i.e., contractile tissue) but also of non-contractile tissues, such as adipose,

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connective, and neurovascular tissues, which co-exist with and support the contractile tissue (Frontera and Ochala, 2015). Because these non-contractile tissues increase with aging (Csapo et al., 2014; Hioki et al., 2020; Kent-Braun et al., 2000; Mikkelsen et al., 2017; Overend et al., 1992; Pinel et al., 2021) and inactivity (Marcus et al., 2010; Ogawa et al., 2021; Taaffe et al., 2009), it is assumed that the accumulation of non-contractile tissues is one factor causing the changes in muscle quality observed in older individuals. However, most studies examining age-related changes in muscle quality have not distinguished between contractile and non-contractile areas (Akima et al., 2001; Kasai et al., 2015; Miller et al., 2021; Ogawa et al., 2012), and the difference in 'pure' muscle quality (i.e., torque/contractile area) remains to be fully elucidated. Previous studies have shown that quadriceps muscle quality declines with aging in males, but not in females (Akima et al., 2001), and the age-related decline in muscle quality was shown in the hamstrings, but not in the quadriceps in males (Ogawa et al., 2012). These findings suggest that muscle quality varies by sex and muscle group. Therefore, a more detailed and exhaustive study is needed to clarify the existence of the age-related change in muscle quality.

As mentioned above, since intramuscular non-contractile tissues increase with aging, we may be overlooking an important morphological factor that can determine age differences in muscle quality. Therefore, the aim of this study was to clarify the differences in muscle composition (contractile and non-contractile tissues) and muscle quality of the quadriceps and hamstrings by age and sex. To investigate this, first, we analyzed the cross-sectional areas (CSA) of contractile and non-contractile tissues and the proportion of non-contractile tissues for muscle areas of the quadriceps and hamstrings muscles. Then, we investigated whether the age and sex differences in muscle quality would be altered when non-contractile tissues were taken into account.

## 2. Methods

### 2.1. Study design and participants

This study employed a cross-sectional observational design that was conducted at Waseda University (Tokorozawa campus) in Japan in 2017. Eighty-two older individuals (41 females, 41 males) and 64 young individuals (32 females, 32 males) participated in this study (Table 1). The recruiting procedure of older individuals is similar to a previous study in our group (Saito et al., 2016). The inclusion criteria were being healthy and having no serious cardiovascular, metabolic, and immunologic disorders and no orthopedic abnormality. They were not taking medication that could affect composition or strength and were relatively health-conscious and active for their age group as their BMI indicates (<25 kg/m<sup>2</sup> for both females and males, Table 1). The young individuals were also healthy and had exercise habits ranging from recreational to competitive level. Thus, both the older and young participants were relatively active and only two participants had a BMI > 30 kg/m<sup>2</sup> (one

**Table 1**  
Participants characteristics.

	Older		Young	
	Female (n = 41)	Male (n = 41)	Female (n = 32)	Male (n = 32)
Age (years)	73.0 ± 6.0 <sup>a</sup>	72.7 ± 5.0 <sup>a</sup>	19.8 ± 4.7	19.9 ± 4.3
Body height (cm)	151.7 ± 5.4 <sup>a,†</sup>	166.4 ± 4.7 <sup>a</sup>	162.0 ± 7.3 <sup>†</sup>	172.3 ± 7.2
Body mass (kg)	52.3 ± 8.0 <sup>†</sup>	65.5 ± 10.9	57.2 ± 8.6 <sup>†</sup>	68.9 ± 10.5
BMI (kg/m <sup>2</sup> )	22.8 ± 4.0	23.7 ± 3.8	21.7 ± 2.1	23.1 ± 2.4

<sup>a</sup> Significant difference between older and young individuals within each sex.

<sup>†</sup> Significant difference between females and males within each age group. *P* < 0.05.

older female and one older male). This study was approved by the Institutional Human Research Ethics Committee. Prior to any measurements, all participants provided written informed consent. Then, magnetic resonance (MR) imaging for the right thigh was performed, followed by maximal voluntary isometric strength measurements.

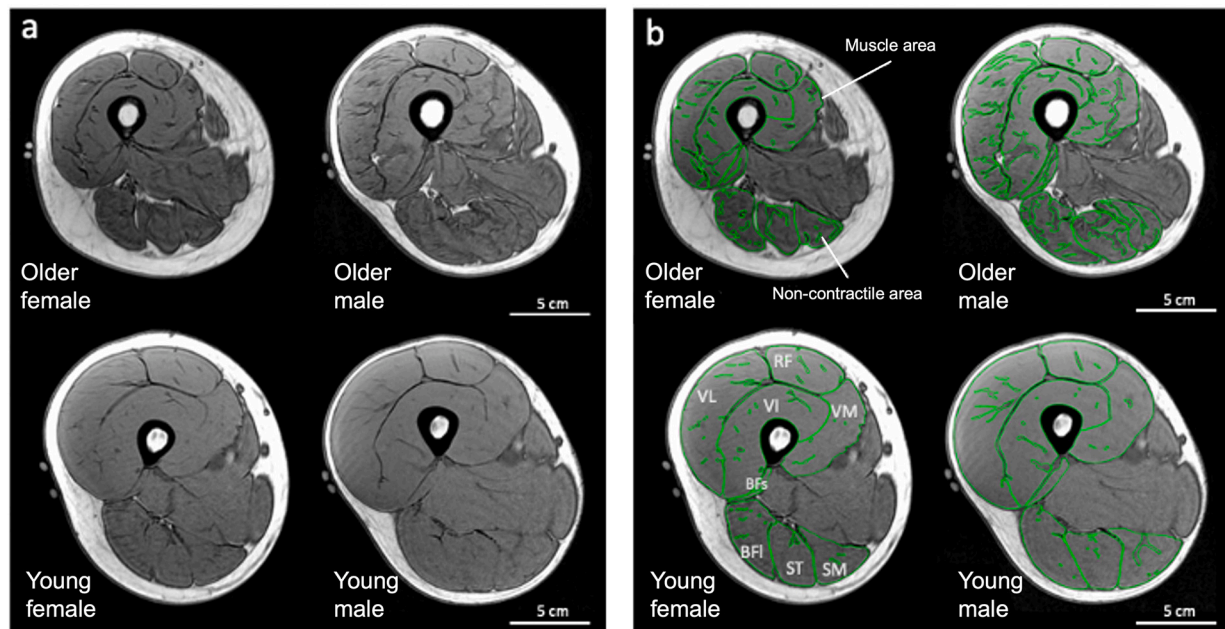
### 2.2. MR imaging

T1-weighted gradient echo MR images (echo time: 3.3 ms, repetition time: 7.8 ms, matrix: 256 × 192, field of view: 240 mm, slice thickness: 8 mm, gap: 0 mm) were obtained using an MR scanner (Signa EXCITE 1.5T, GE Medical Systems, Chicago, USA). The MR images of right thigh in the transverse plane were taken using 8-channel body array coil (GE Medical Systems; Chicago, USA). This protocol has been used for evaluating muscle size in large population studies (Culvenor et al., 2016; Maden-Wilkinson et al., 2014), so we adopted this conventional sequence to evaluate whole muscle tissue. Before the scanning, an oil capsule was put at 50 % of the thigh length on the lateral side of right leg. The participants lay supine with their legs fully extended and relaxed in the magnet bore.

The CSAs of muscles and intramuscular non-contractile tissues of each of individual quadriceps (VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris) and hamstrings (BFs, biceps femoris short head; BFL, biceps femoris long head; ST, semitendinosus; SM, semimembranosus) at midthigh were measured using imaging analysis software (OsiriX medical imaging software, Pixmeo, Geneva, Switzerland) (Fig. 1). The CSA at midthigh is most closely correlated with the amount of muscle ( $R^2 = 0.970$ ) and intramuscular fat ( $R^2 = 0.979$ ) tissues in the whole thigh (Hogrel et al., 2015). Contractile tissue CSA was calculated by subtracting non-contractile tissues from the muscle area. The sum of the CSA of each individual muscle of the same muscle group was taken as the CSA of the muscle group. Intramuscular non-contractile tissues were distinguished from contractile tissue by manual tracing according to a previous study (Mikkelsen et al., 2017) and the boundary with contractile tissue was determined as follows. On T1-weighted images, fat tissue generally appears as high signal intensity (white tissue), while hard tissues rich in collagen fibers such as bone, ligament, and tendon tissues appear as low signal intensity (black tissue) (Maden-Wilkinson et al., 2014; Orgiu et al., 2016). However, due to the imaging parameters of this study, the chemical shift results in low signal areas by canceling the signals of co-located fat and water in muscles (R. H. Hashemi and W. G. Bradley, 2017). Thus, it was possible to distinguish collagen fiber or fat from contractile tissue (gray tissue) based on signal intensity, and the low-signal areas were defined as intramuscular non-contractile tissues. Intramuscular non-contractile tissues are included via neurovascular tissues and construct a connective tissue network (Purslow, 2020). Considering this network structure composed of various non-contractile tissues, the non-contractile tissues (low-signal areas) for which we could confirm continuity from the serial MR images were traced. The segmentations were conducted manually by one trained observer.

### 2.3. Knee extension and flexion torque

Maximal voluntary isometric knee extension and flexion torque was measured in random order using a custom-made dynamometer for measuring isometric contraction (VTK-002, Vine, Japan). Participants sat on the seat with hip and knee joint angles of 80° and 70° (anatomical position = 0°), respectively. Previous studies have been reported that the peak isometric knee extension torque occurs at ~70° knee joint angle (Pincivero et al., 2004). Although the isometric knee flexion peak torque occurs at a more extended angle (Mohamed et al., 2002), changing the knee joint angle is time consuming, therefore the knee joint angle (70°) was fixed for the present measurement as a priority to refrain from the burden of excessive restraint time on the measurement, especially in the older participants. The pelvis was secured to the backrest of



**Fig. 1.** Examples of T1-weighted MR images of the midthigh from older and young individuals. Note: green solid line shows the outline of each tissue of the thigh. VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius; RF, rectus femoris; BF, biceps femoris short head; BFL, biceps femoris long head; ST, semitendinosus; SM, semimembranosus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the dynamometer with non-elastic band to restrict any upper body movement. The ankle strap on the arm of dynamometer was attached proximal to the lateral malleolus of the right leg. The arm was connected to a calibrated torque sensor with internal strain gauges and the torque signals were amplified by a strain amplifier (DPM-711B, Kyowa, Japan) and converted (Power Lab, AD Instruments, Australia) to a computer at a sampling frequency of 1000 Hz with a low-pass filter (cut-off frequency, 10 Hz). Prior to maximal testing, the participants performed submaximal contractions at 30, 50, and 70 % of maximal intensity as familiarization and warm up. The peak torque during maximal contraction was measured twice. Each maximal contraction lasted for ~3 s, and a minimum of 1-min rest was allowed between contractions. If the difference between the two peak values was >10 %, additional measurements were performed until the difference between the highest and second highest values was <10 %. The highest value among those measurements was adopted as the peak torque.

#### 2.4. Data analysis

The proportion of non-contractile tissues in muscle area of each quadriceps and hamstrings was calculated by dividing the non-contractile CSA of each muscle group by the muscle CSA (Non-contractile/muscle CSA). As an index of muscle quality, the peak knee extension and flexion torque was divided by the muscle or contractile CSA ( $MQ_{MT}$  and  $MQ_{CT}$ ) of the quadriceps and hamstrings, respectively.

#### 2.5. Statistical analysis

All statistical analyses were performed using SPSS statistical software (IBM SPSS Statistics 27, IBM, USA). We used the Levene's test to examine the equality of variance between groups, and the equalities in many variables (Quadriceps muscle CSA, Quadriceps contractile CSA, Quadriceps non-contractile CSA, Quadriceps non-contractile/muscle CSA, Hamstrings muscle CSA, Hamstrings contractile CSA, Hamstrings non-contractile CSA, Hamstrings non-contractile/muscle CSA, Knee extension torque and knee flexion torque) were rejected. When the variances are not equal and the sample sizes differ between independent groups, the Welch's *t*-test provides a better control of Type 1 error rates

than the Student's *t*-test (Delacre et al., 2017). Thus, the Welch's *t*-test with Bonferroni's correction (Fagerland, 2012) was performed to examine the difference between age (older females vs. young females, older males vs. young males) and sex (older females vs. older males, young females vs. young males) in each variable, instead of applying ANOVA. Other than the Scheffe test, it is not necessarily true that the pairwise comparisons are not significant if the ANOVA is not significant, and thereby Ruxton and Beauchamp (2008) highlighted the logical flaw on the commonly adopted policy of first performing an ANOVA and only investigating post hoc comparisons if the ANOVA is significant. Thus, we did not adapt ANOVA in our case. A paired *t*-test was used to compare quadriceps and hamstrings non-contractile/muscle CSA within the same subjects. Cohen's *d* was calculated as indices of effect size for the Welch's *t*-test and paired *t*-test. All data are presented as means and standard deviation. The significance level for all statistical tests was set at 0.05.

### 3. Results

#### 3.1. Muscle composition and strength

The CSA of muscle and contractile tissue of the quadriceps was greater in young than in older individuals ( $P < 0.001$ ,  $d = 2.402$ – $2.951$ ) and in males than in females ( $P < 0.001$ ,  $d = 0.976$ – $2.138$ ). The non-contractile CSA of the quadriceps was greater in older than in young individuals ( $P < 0.001$ ,  $d = 0.983$ – $1.171$ ) and did not present sex differences. In the hamstrings, the CSA of muscle and contractile tissue was greater in young than in older individuals ( $P < 0.001$ ,  $d = 1.598$ – $2.705$ ) and in older males than in older females ( $P < 0.001$ ,  $d = 0.785$ – $1.749$ ) but did not show the sex difference in the young groups. Non-contractile CSA of hamstrings was greater in older than in young individuals ( $P < 0.001$ ,  $d = 1.146$ – $1.534$ ) and did not present sex differences. The torque of both knee extension and flexion was lower in the older than in the young individuals ( $P < 0.001$ ,  $d = 2.113$ – $3.165$ ), and in females than in males ( $P \leq 0.009$ ,  $d = 1.431$ – $2.224$ ) (Table 2).

**Table 2**  
Muscle, contractile and non-contractile CSA and muscle strength.

	Older		Young	
	Female (n = 41)	Male (n = 41)	Female (n = 32)	Male (n = 32)
Quadriceps muscle CSA (cm <sup>2</sup> )	37.4 ± 7.2 <sup>a,†</sup>	52.8 ± 7.6 <sup>a</sup>	64.6 ± 15.1 <sup>†</sup>	77.7 ± 11.3
Quadriceps contractile CSA (cm <sup>2</sup> )	34.1 ± 6.8 <sup>a,†</sup>	48.7 ± 6.9 <sup>a</sup>	62.5 ± 14.6 <sup>†</sup>	75.4 ± 11.3
Quadriceps non-contractile CSA (cm <sup>2</sup> )	3.2 ± 1.1 <sup>a</sup>	4.1 ± 1.8 <sup>a</sup>	2.2 ± 1.1	2.3 ± 1.1
Hamstrings muscle CSA (cm <sup>2</sup> )	18.8 ± 3.4 <sup>a,†</sup>	25.2 ± 4.0 <sup>a</sup>	28.8 ± 5.3	33.3 ± 6.2
Hamstrings contractile CSA (cm <sup>2</sup> )	16.7 ± 3.3 <sup>a,†</sup>	22.3 ± 3.7 <sup>a</sup>	27.8 ± 5.0	32.3 ± 6.1
Hamstrings non-contractile CSA (cm <sup>2</sup> )	2.1 ± 1.2 <sup>a</sup>	2.9 ± 1.4 <sup>a</sup>	1.0 ± 0.8	1.0 ± 0.8
Knee extension torque (Nm)	102.2 ± 24.2 <sup>a,†</sup>	154.8 ± 28.3 <sup>b</sup>	178.3 ± 38.0 <sup>†</sup>	252.2 ± 63.4
Knee flexion torque (Nm)	36.5 ± 7.9 <sup>a,†</sup>	62.2 ± 14.2 <sup>b</sup>	73.0 ± 15.0 <sup>†</sup>	104.3 ± 25.2

<sup>a</sup> Significant difference between older and young individuals within each sex.

<sup>†</sup> Significant difference between females and males within each age group.  $P < 0.05$ .

### 3.2. Intramuscular non-contractile tissues

Fig. 2 shows the non-contractile/muscle CSA of the quadriceps and hamstrings in each age and sex group. This was significantly greater in the older than in the young individuals for both the quadriceps ( $P < 0.001$ ,  $d = 1.940$ – $2.603$ ) and hamstrings ( $P < 0.001$ ,  $d = 1.698$ – $2.044$ ). No sex differences were shown in either muscle group. The non-contractile/muscle CSA of the hamstrings was significantly greater than that of the quadriceps in the older groups ( $P \leq 0.002$ ,  $d = 0.524$ – $0.948$ ), with no significant differences between the sex groups (Fig. 2).

### 3.3. Muscle quality of quadriceps and hamstrings

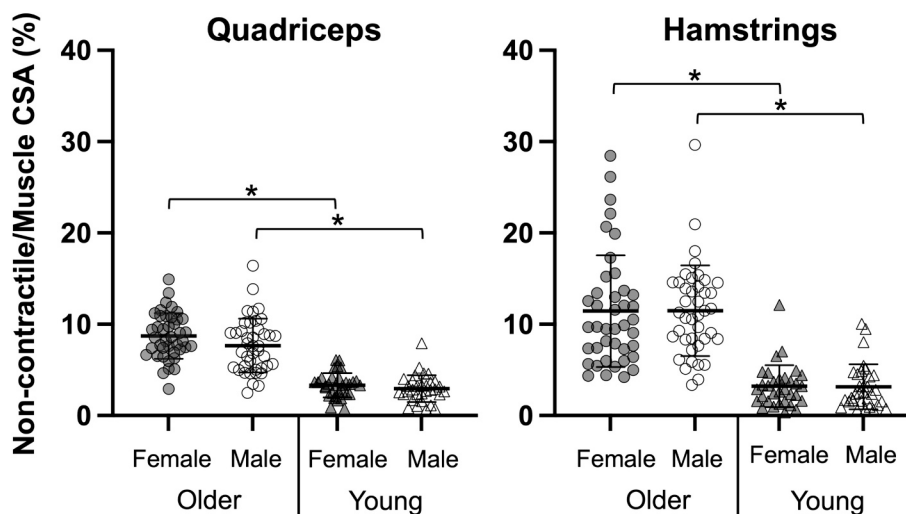
For the quadriceps muscles, there were no significant differences in muscle quality between age groups or sexes for  $MQ_{MT}$  and  $MQ_{CT}$  (Fig. 3A&B). In contrast, for the hamstrings,  $MQ_{MT}$  was significantly lower in the older individuals than in the young individuals ( $p \leq 0.001$ ,  $d = 0.231$ – $1.060$ , Fig. 3C) and that of females was lower than that of males in both older ( $P = 0.004$ ,  $d = 0.270$ ) and young groups ( $P = 0.012$ ,  $d = 0.966$ ). The age-related differences disappeared for  $MQ_{CT}$ , whereas the values of females were still lower than those of males after the correction in both age groups ( $P < 0.029$ ,  $d = 0.889$ – $0.923$ , Fig. 3D).

## 4. Discussion

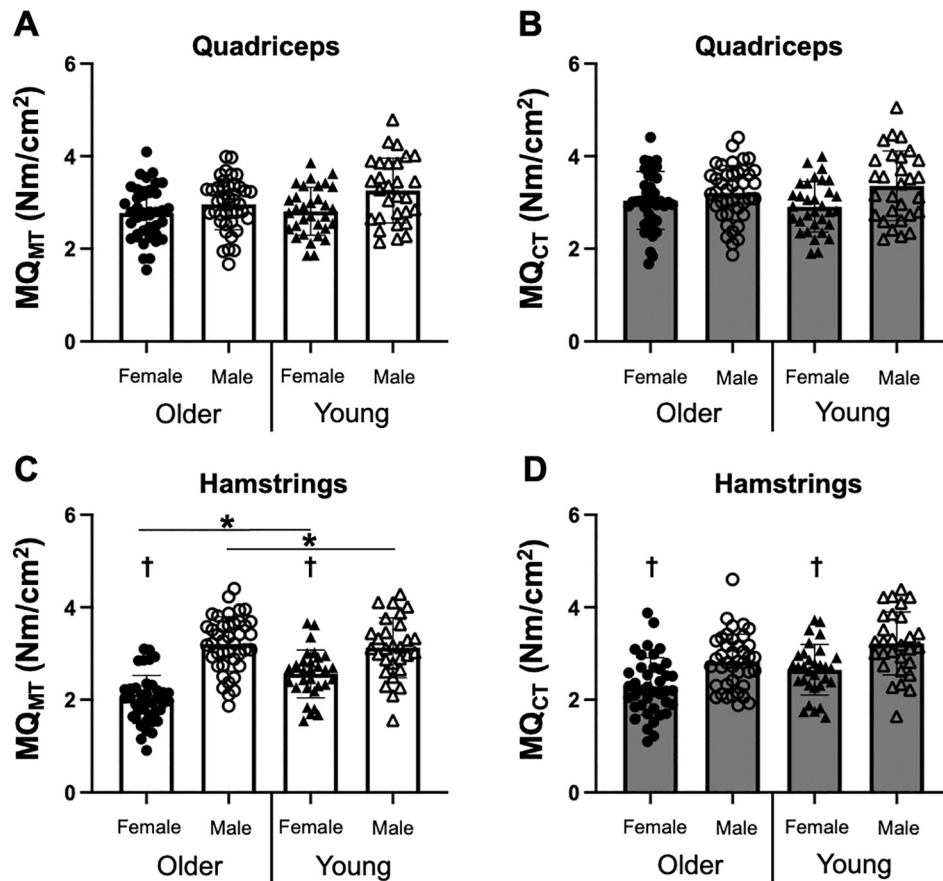
Muscle quality corrected for non-contractile tissues ( $MQ_{CT}$ ) was not significantly different between older and young individuals, although

the conventional muscle quality ( $MQ_{MT}$ ) showed the age differences in the hamstrings. This result indicates that the torque generating capacity of contractile tissue itself is not largely different between older and young individuals. Thus, the conventional evaluation of muscle quality (i.e., torque/muscle CSA) (Francis et al., 2017b; Morse et al., 2004) can underestimate the torque generating capacity of muscle especially in older individuals, and this supports the importance of considering changes in muscle composition in skeletal muscle with aging. The phenomenon of age-related decline in muscle quality and its possible causes (e.g., muscle architecture, specific tension of muscle fibers, non-contractile tissues and muscle recruitment) have been previously reported (Akima et al., 2001; Macaluso et al., 2002; Morse et al., 2004). We have observed that the age differences in muscle quality disappear at the corrected indicator ( $MQ_{CT}$ ), which further suggests that the accumulation of non-contractile tissues is one important factor causing the age-related decline in muscle quality.

The presence of non-contractile tissues was greater in older than in young individuals, and the age differences were more pronounced in the hamstrings (Fig. 2). Previous studies have also reported that the extent of the accumulation of intramuscular non-contractile tissues of older individuals is greater in the hamstrings than in the quadriceps (Akima et al., 2015; Overend et al., 1992), suggesting the muscle specificity of the age-related increase in non-contractile tissues. Looking back at the results of muscle quality, no age differences were observed in the quadriceps in either  $MQ_{MT}$  or  $MQ_{CT}$ , and the hamstrings showed age-related differences in  $MQ_{MT}$ , but not in  $MQ_{CT}$ . When calculating muscle quality using muscle CSA ( $MQ_{MT}$ ), which does not distinguish between contractile and non-contractile tissues, the more non-contractile



**Fig. 2.** The proportion of intramuscular non-contractile tissues in muscles of quadriceps and hamstrings muscles. Note: \*Significant difference between older and young individuals.  $P < 0.05$ .



**Fig. 3.** Muscle quality of quadriceps and hamstrings muscles. Note: \*Significant difference between older and young individuals within each sex. †Significant difference between females and males within each age group.  $P < 0.05$ . MQ<sub>MT</sub>, torque/muscle CSA, MQ<sub>CT</sub>, torque/contractile CSA.

tissues that do not directly contribute to force generation, the lower the torque generating capacity of ‘pure’ muscles (i.e., contractile tissue) should be estimated. As additional data, the correlation between non-contractile/muscle CSA and MQ<sub>MT</sub> (or MQ<sub>CT</sub>) was tested, and the non-contractile/muscle CSA of the hamstrings only showed a significant negative correlation with MQ<sub>MT</sub> when all groups were combined ( $r = -0.32$ ,  $P < 0.001$ ). Although previous findings on the age-related changes are inconsistent, the presence of non-contractile tissues can be one of the factors overestimating the age differences in muscle quality. However, the participants of this study were relatively healthy and active for their age group and these results may not apply to a population with lower physical activity levels. In less active population, the age-related differences in MQ<sub>CT</sub> may be presented due to the intrinsic changes in contractile tissue (e.g., fiber type composition and neural drive). Additionally, the accumulation of non-contractile tissues should be taken into account even when discussing the muscle specificity of muscle atrophy. Previous studies, that have not considered intermuscular differences in non-contractile tissue distribution, have often focused on the quadriceps to capture the aging muscle atrophy in the lower limb, because the cross-sectional studies of older (~75 yr) and young (~25 yr) individuals have reported greater muscle atrophy of the quadriceps than that of hamstrings (Naruse et al., 2023b). However, the present study found a significantly greater accumulation of non-contractile tissues in the hamstrings (11.5 %) than in the quadriceps (8.2 %) in older individuals. Moreover, a 5-year longitudinal study in septuagenarians have observed the greater muscle atrophy of the hamstrings (-5.9 %) compared to the quadriceps (-3.3 %) (Naruse et al., 2023a). These findings highlight the importance of hamstrings to assess the age-related muscle atrophy in lower limb.

Although there were no age differences in muscle quality when

corrected for non-contractile tissues (MQ<sub>CT</sub>), the hamstrings showed a significant sex difference (males > females) in both age groups (Fig. 3). Moreover, there were no sex differences in the presence of non-contractile tissues within the hamstrings (Fig. 2). These findings suggest the sex differences in muscle quality are not related to muscles’ morphological factors such as the amount of contractile and non-contractile tissues, but to the functional factors of skeletal muscles. Joint torque generation capacity is known to be influenced by the extent of neural adaptation as well as muscle size (Aagaard, 2003; Häkkinen et al., 1985; Moritani, 1993), which may influence the sex differences in muscle quality. A previous study has observed sex differences in muscle activation of hamstrings muscles, with no sex differences in that of quadriceps muscles during different exercises for quadriceps and hamstrings (front lunge, side lunge and bilateral squat) in older population (Followay et al., 2023). Additionally, other previous studies have also suggested that females are quadriceps dominant and males are hamstrings dominant during single-leg squat in young populations (Youdas et al., 2007; Zeller et al., 2003). The quadriceps and hamstrings muscles are frequently activated during many daily living activities (e.g. walking, maintaining postural stability and going up and down the stairs), and thus our findings of sex differences in muscle quality may reflect the differences in the contribution of muscle activation between sexes.

This study has some limitations that need to be considered. Firstly, due to the problem of signal cancellation on MR images, the contractile tissue around areas of signal cancellation might have been traced as non-contractile area. In addition, this imaging protocol did not enable the segmentation of non-contractile tissues into each component such as adipose, connective and neurovascular tissues. To better elucidate the age-related change in muscle composition, a future study should divide

and evaluate each non-contractile tissue component. Secondly, the present study measured the anatomical CSA at midthigh. In pennate muscles, anatomical CSA, unlike physiological CSA, does not cover all muscle fibers and the force/torque per physiological CSA is typically used to evaluate muscle quality. However, a recent study has reported that anatomical CSA at midthigh is more strongly correlated with muscle force than physiological CSA in the quadriceps (Balshaw et al., 2021), and thus the torque normalized to anatomical CSA should be also useful for estimating muscle quality. Moreover, some previous studies have used torque per muscle volume as an index with the same dimension as that of torque per physiological CSA (Fukunaga et al., 2001; Macaluso et al., 2002; Morse et al., 2004). The regions of high fat accumulation in the thigh vary by muscle group (e.g., central in the quadriceps but distal in the hamstring), and the impact of aging might be region-specific (Yoshiko et al., 2017). Considering the inhomogeneous distribution of non-contractile tissues, future studies should analyze muscle composition and estimate muscle quality by volume. Thirdly, both isometric knee extension and flexion torques were measured at 70° of knee joint angle. While this was the optimal angle for peak torque in knee extension (Pincivero et al., 2004), a more extended position is preferred for knee flexion (Mohamed et al., 2002). In order to further explore the differences in muscle quality between the muscle groups (i.e., quadriceps vs hamstrings), future studies should use the peak torque value exerted at the optimal joint angle for each joint movement. Finally, we could not measure the neural activation during the maximal voluntary isometric contraction. Previous studies (Macaluso et al., 2002; Morse et al., 2004) reported that lower muscle quality in older individuals was associated with an increased coactivation of antagonist muscles. Therefore, it remains unclear whether our findings of age and sex differences in muscle quality were attributed to the variation of neural activation.

In summary, this study evaluated muscle composition (the ratio of contractile to non-contractile tissue) of the quadriceps and hamstrings muscles in each age and sex group and examined the influences of two different muscle quality's indices (MQ<sub>MT</sub> vs MQ<sub>CT</sub>) on their age and sex differences. The presence of non-contractile tissues was greater in older individuals compared to young counterparts, and it was greater in the hamstrings than in the quadriceps in older individuals. When corrected for intramuscular non-contractile tissues, muscle quality (MQ<sub>CT</sub>) of the hamstrings did not present the age differences, even though the conventional muscle quality (MQ<sub>MT</sub>) differed between groups. These findings imply that the age-dependence of muscle quality result from an extensive increased presence of non-contractile tissues, but the sex differences in muscle quality may be related to other factors such as neural activation.

#### CRediT authorship contribution statement

**Hoshizora Ichinose:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Fumiko Tanaka:** Project administration, Data curation. **Takaki Yamagishi:** Writing – review & editing, Investigation, Data curation. **Natsuki Sado:** Writing – review & editing. **Hiroto Shiotani:** Writing – review & editing. **Pavlos E. Evangelidis:** Writing – review & editing, Validation. **Munekazu Naito:** Methodology. **Shigenobu Shibata:** Project administration, Funding acquisition. **Yasuo Kawakami:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no conflicts of interest.

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